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TITLE: Mineralocorticoid receptor compositions and methods

## Detailed Description Text (62):

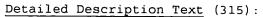
Plasmid phH3 relates to clone lambda hH3 which was isolated from a human heart lambda gtll cDNA library using a nick-translated 700-bp EcoRI-SmaI fragment representing the 5' portion of lambda hKA1 (Clones lambda hKE4 and lambda hKA1 were isolated from a human kidney lambda gt10 cDNA library; see Experimental Section V., H., especially the subsections labeled as FIGS. 48 and 49 and FIGS. 50 and 51 Methods). Clone lambda hH3 carries cDNA which codes for an estrogen related receptor referred to herein as hERR2. The cDNA from phH3 be inserted into pGM3 to create pGERR1, a drawing of which is also shown in FIG. 10. The functional and structural characteristics of receptor-like polypeptides hERR1 and hERR2 are disclosed and discussed in Experimental Section V.

## Detailed Description Text (102):

A library of cDNA clones was constructed in the phage expression vector lambda gtll using poly(A).sup.+ RNA from the human lymphoid cell line IM-9 as template, as described previously (Weinberger et al., (1985a)). This library was initially screened with a rabbit polyclonal antiserum to the purified glucocorticoid receptor, resulting in the isolation of several immunopositive candidate clones from .about.2.5.times.10.sup.5 plaques. The beta-galactosidase fusion proteins generated from these clones were used to affinity-purify receptor epitope-specific antibody, which was subsequently recovered and identified by binding to protein blots of cellular extracts. Three clones containing inserts expressing antigenic determinants of the human glucocorticoid receptor were isolated. The inserts of these clones, although of different sizes, cross-hybridized, indicating that they contained a common sequence which presumably delimits the major immunogenic domain of the receptor. Together, these clones spanned 1.4 kilobase pairs (kbp) but were clearly not long enough to code for the entire receptor, which was estimated to require .about.2,500 nucleotides to encode a polypeptide of M.sub.t 94K.

## Detailed Description Text (103):

To isolate additional cDNA clones we again screened the original library and also examined a second library (given by H. Okayama) prepared with poly(A).sup.+ RNA from human fibroblasts in the vector described by Okayama and Berg, Molec. Cell. Biol., 3: 280-289 (1983). Using one of the immunopositive cDNA inserts (hGR1.2) as a probe, 12 clones were isolated that, together, covered more than 4.0 kbp. The nucleotide sequences of these clones were determined by the procedure of Maxam and Gilbert, Proc. Natl. Acad. Sci. U.S.A., 74: 560-564 (1977), according to the strategy indicated in FIG. 1. RNA blot analysis indicated that a cDNA insert of 5-7 kilobases (kb) would be necessary to obtain a full-length  $\underline{\text{clone}}$  and sequence analysis indicated that the overlapping  $\underline{\text{clones}}$  OB7 and hGR5.16 spanned an open reading frame of 720 amino acids, not large enough to encode the complete receptor. Therefore, a second human fibroblast cDNA library of .about.2.times.10.sup.6 transformants was screened, yielding a clone (OB10) containing a large insert that extended 150 base pairs (bp) upstream of the putative translation initiation site (see FIGS. 1 and 2). Sequence analysis predicts two protein forms, termed alpha and beta, which diverge at amino acid 727 and contain additional distinct open reading frames of 50 and 15 amino acids, respectively, at their carboxy termini (see FIG. 2). The alpha form, represented by clone OB7, is the predominant form of glucocorticoid receptor because eight cDNA clones isolated from various libraries contain this sequence.



One approach to search for unrecognized hormone response systems is to systematically employ reduced stringency hybridization to screen recombinant DNA libraries for novel hormone receptors. The DNA-tau segment of the estrogen receptor was used to initiate these studies. Analysis of the lambda gt10 human testis cDNA library identified 3 positives at a frequency of one clone per 3.times.10.sup.5 recombinant phages. Nucleotide sequence analysis revealed that two of these clones actually encode the estrogen receptor while the third one spanning 2.0 kilobases and named lambda hT16, showed only partial sequence homology. In turn, this clone was used to screen human fetal kidney and adult heart cDNA libraries, resulting in the identification of 3 additional clones. Both clones from the kidney library, lambda hKE4 and lambda KA1, represent the same gene product as lambda hT16 while the cardiac clone, lambda hH3, is only partially related. The composite sequence of the three cDNAs sharing identical sequences, herein referred to as hERR1, is shown in FIG. 49. Assuming a poly(A) tail of .about.150-200 nucleotides (Sawiki et al., J. Mol. Biol., 113: 219-235 (1977)), this sequence (.about.2430 nt) must be nearly full length. The cDNA insert from lambda hKA1 contains nucleotide 179 to 2430 while lambda hKE4 represent a rare messenger RNA splicing error with deletion of exon 2 and insertion of intron sequences. The exon/intron boundaries suggested by lambda hKE4 were confirmed by cloning and partially sequencing the genomic fragments encoding this gene (data not shown). The sequence surrounding the first ATG agrees with the consensus described by Kozak, M., (1984) for a translation initiation site. An open reading frame of 521 amino acids predicting a polypeptide of Mr 57300 is flanked by a 775 nucleotide 3'-untranslated region.